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RESEARCH PAPER

Miniaturised enzymatic conductometric biosensor with Nafion membrane for the direct determination of formaldehyde in water samples

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Abstract A new conductometric enzyme-based biosensor was developed for the determination of formaldehyde (FA) in aqueous solutions. The biosensor was prepared by cross-linking formaldehyde dehydrogenase from *Pseudomonas putida* with bovine serum albumin in saturated glutaraldehyde vapours (GA) at the surface of interdigitated gold microelectrodes. Nicotinamide adenine dinucleotide cofactor (NAD^+) was added in solution at each measurement to maintain enzyme activity. Addition of a Nafion layer over the enzyme modified electrode resulted in a significant increase of biosensor signal due to enhanced accumulation of protons generated by enzymatic reaction at the electrode surface. Different parameters affecting enzyme activity or playing a role in ionic transfer through the Nafion membrane were optimised. In optimal conditions (0.045 mg enzyme, 30 min exposure to GA, 0.3 μL of a 1 % (*v/v*) Nafion solution deposit, measurement in 5 mM phosphate buffer pH 7 containing 20 μM NAD^+), the biosensor signal was linear up to 10 mM FA, and the detection limit was 18 μM. Relative standard deviations calculated from five consecutive replicates of FA solutions were lower than 5 % in the 1–10 mM range. The biosensor was successfully applied to the determination of FA in spiked water samples (tap water and Rhone river water), with recoveries in the 95–110 % range.

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Introduction

With 20 million tons produced worldwide annually, formaldehyde (FA) is one of the most important commercialised chemical products. FA is involved as intermediary reactant in the synthesis of several chemicals and is used as adhesive or binder in wood, paper and textiles, as germicide, insecticide or fungicide in agriculture, as well as preservative in goods or as sterilising agent for medical applications [1]. Outgassing from resins in furniture, wallpapers or paints is a major indoor FA source of emission. Adverse effects on central nervous and immune systems have been observed in humans following acute expositions to high concentrations of this compound, while respiratory disease or allergic dermatitis are associated with chronic exposure to lower levels. FA has been also classified as human carcinogen by the US Environmental Protection Agency and the International Agency for Research on Cancer and is suspected to be toxic to human development and reproduction [2]. Analytical methods used for formaldehyde determination are mostly based on spectrofluorometric [3] and chromatographic [4–7] techniques. These techniques do not all offer the adequate selectivity and sensitivity or require toxic reagents and/or cumbersome instrumentation not suitable for in situ measurements.

In this context, electrochemical biosensors appear as very attractive alternatives, providing fast, selective, sensitive and real-time response to many pollutants [8, 9]. Although some whole-cell biosensors have been reported for FA detection [10], enzymes are more widely used as bioreceptors. Formaldehyde dehydrogenase (FDH) [11–21] and, in a lesser extent, alcohol oxidase (AOX) [22–25] have been proposed

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as sensing elements for the construction of FA biosensors. AOX, which belongs to the oxidoreductase enzymes family, catalyses primarily the oxidation of low-molecular-weight alcohols, especially methanol, using oxygen as cofactor. Catalytic activity of AOX towards aldehydes is much lower [26]. Therefore, AOX-based biosensors offer lower sensitivity and selectivity compared with FDH-based devices. Besides, AOX is less stable than FDH [27].

For its part, FDH belongs to the zinc alcohol dehydrogenase family and is produced by a wide variety of microorganisms. FDH catalyses FA oxidation into formic acid, generally using nicotinamide adenine dinucleotide (NAD^+) as electron acceptor, while NAD^+ is reduced to NADH (Eq. 1):

$$HCHO + NAD^+ + H_2O \rightarrow HCOO^- + NADH + 2H^+ \quad (1)$$

FA electrochemical biosensors mostly imply amperometric transduction, and NADH produced is reoxidised at the electrode surface, enabling both NADH detection and NAD^+ recycling. However, the optimal overpotential value to be applied for electrochemical oxidation of NADH is high, approximately 0.6 V at pH 7, resulting in interfering reactions, electrode fouling and the formation of an enzymatically inactive form of NAD^+. Chemical redox mediators or nanomaterials such as carbon nanotubes may be added to solve this problem [13, 15–17, 20].

Different types of FDH have been also used as bioreceptors. Recombinant FDH extracted from *Hansenula polymorpha* offers the advantage to be more stable than commercially available FDHs from *Pseudomonas putida* bacteria, as well as *Candida bodiini* or *Pichia pastoris* yeast strains [11, 12]. However, production of recombinant enzymes is costly and laborious. FA oxidation catalysed by FDHs issued from *C. bodiini,* and *H. polymorpha* requires the participation of an additional external cofactor, glutathione (GSH). Therefore, commercial GSH-independent FDH from *P. putida* (PFDH) is the most common enzyme used in the elaboration of FA biosensors. NAD^+ is known to bind to the active site of free PFDH through a strong but non-covalent bonding favouring catalytic activity [28]. Hence, enzyme immobilisation on the electrode must be carefully optimised, not only to preserve the native conformation of the biomolecule, but also to favour the approach and fixation of NAD^+ to PFDH active site. Two strategies that consist in NAD^+ and PFDH co-immobilisation on the transducer [12, 17] or NAD^+ addition in solution after immobilisation of the enzyme on the electrode [13, 15, 18, 20, 21] have been reported.

In this work, we propose a sensitive and miniaturised conductometric biosensor for FA determination in water samples. The biosensor was prepared by first immobilising PFDH at the surface of gold interdigitated microelectrodes through crosslinking with glutaraldehyde vapours and then covering the biological layer by a Nafion membrane. Nafion is a negatively charged fluorinated polymer offering attractive properties as ionic conductor and high selectivity towards anionic interfering substances, e.g. ascorbate [29]. Various enzymes have been successfully immobilised using this material for biosensor application [21, 30–36]. However, to the best of our knowledge, this is the first time that a biosensor based on cross-linked PFDH trapped behind a Nafion membrane and a conductometric mode of transduction is proposed for FA analysis.

Experimental

Reagents

PFDH (EC 1.2.1.46, 4 U mg^{-1} solid extracted from *P. putida* sp, bovine serum albumin (BSA), glutaraldehyde (GA) (grade II, 25 % aqueous solution), β-NAD^+ (98 % from yeast), Nafion (5 wt% in a mixture of aliphatic alcohols and water containing 15–20 % H_2O), KH_2PO_4 (>99 %), K_2HPO_4 (98 %), paraformaldehyde powder (95 %) and NaOH pellets (>98 %) were purchased from Sigma-Aldrich. Ethanol (99.7 %) and methanol (99.8 %) were from Carlo Erba.

Preparation of solutions

All aqueous solutions were prepared using 18 MΩ cm ultrapure water from an Elgastat UHQ II purification system (Elga LabWater, Le Plessis Robinson, France).

The 1 wt% Nafion solution was prepared by dilution of the commercial 5 wt% solution in ultrapure water and then neutralised to pH 7–9 with 1 M NaOH.

A 2 M FA stock solution was prepared by hydrolysing paraformaldehyde. For that, 0.3 g of the polymer was suspended in 5 mL of ultrapure water containing 30 μL of 1 M NaOH, and the mixture was maintained for about 4 h at 65 °C until complete clarification. FA concentration in the stock solution was controlled by titrimetry after reaction with sodium sulphite. This solution, kept at 4 °C for a maximum of 1 week, was used for the daily preparation of diluted standards in 5 mM phosphate buffer pH 7.

For biosensors construction, 20 mM phosphate buffer solutions of pH 7.1 containing either 50 g L^{-1} BSA, 100 g L^{-1} glycerol, 150 g L^{-1} PFDH and 65 g L^{-1} NAD^+ ("enzyme solution") or a mixture of 200 g L^{-1} BSA, 100 g L^{-1} glycerol and 65 g L^{-1} NAD^+ ("BSA solution") were prepared just before the first utilisation and stored at −20 °C until further use. Glycerol is a powerful cryoprotectant able to keep proteins conformation safe at low temperatures [37].

Sensor design

The conductometric transducers (5×30 mm) consist of two pairs of interdigitated gold electrodes (sensitive area of each

electrode ~1 mm^2; thickness of the gold layer, 150 nm), the first one being used as the reference electrode and the second one as working electrode. They were manufactured in Lashkaryov Institute of Semiconductor Physics (Kiev, Ukraine) by deposition on a ceramic substrate (AlO_3). An intermediate layer of chromium (0.1 nm thick) was used for a better adhesion of gold. Digits are about 1 mm long, while digits and interdigital spaces are about 20 μm [38].

Biosensor construction

As a differential experimental setup was used, 0.3 μL of the enzyme solution was pipetted onto the working pair of electrodes, while the same volume of BSA solution was dropped onto the reference electrodes. The solutions were allowed to evaporate for 30 min at room temperature. Then, (1) 0.3 μL of the aqueous Nafion solution was pipetted onto both reference and working electrodes (Fig.1a), or (2) the sensor chip was placed for 30 min in a saturated GA vapour atmosphere (Fig.1b), or (3) both pairs of electrodes were covered by Nafion membrane after cross-linking with GA (Fig. 1c).

Whatever the immobilisation method used, the electrodes were allowed to dry for 1 h after fabrication, stored overnight at 4 °C, reconditioned in 5 mM phosphate buffer for 30 min before the first measurement and further stored at 4 °C in 20 mM phosphate buffer pH 7.

Electrochemical measurements

Microelectrodes were placed in a glass cell filled with 5 mL of a 5 mM phosphate buffer pH 7 under magnetic stirring.

For conductometric measurements, an alternating voltage (10 mV amplitude, 100 kHz frequency) generated by a low-frequency waveform generator (SR830 Lock-in amplifier from Stanford Research Systems) was applied to the differential pairs of electrodes, and the resulting difference of conductance ΔG was measured between the working and the reference pairs. Then, 50 μL of a 2 mM NAD^+ stock solution was pipetted into the electrolyte, and the differential conductance was allowed to stabilise. Subsequently, small aliquots (typically 5 to 120 μL) of concentrated solutions containing FA were added into the vessel, and the biosensor response, corresponding to the evolution of ΔG after FA addition, was recorded. Measurements were performed at 23±2 °C. Initial rates of reaction were calculated from the first 30 s linear portion of the conductometric response. The background noise (N) was measured in the stable part of the response just before injection. The detection limit was calculated as FA concentration generating a ΔG/N of 3.

Electrochemical impedance spectroscopy measurements were performed in the same conditions of electrolyte and temperature using a Voltalab 80 instrument (Radiometer Analytical, Villeurbanne, France) and a two-electrode configuration. A 10-mV amplitude alternating potential was applied to the working electrode, the frequency ranging from 100 mHz to 100 kHz and 5 points per decade being recorded.

Results and discussion

PFDH mode of immobilisation

The retention of enzyme conformation and specific activity is a key issue in biosensor development, which strongly depends on the way the protein is immobilised on the transducer [39]. This problem is particularly complex for multimeric enzymes (e.g. dehydrogenases, oxidases, catalases, aldolases) that may easily deactivate by subunit dissociation [40]. Belonging to the dehydrogenase family, PFDH is a homotetramer, each subunit containing two domains, the first one for NAD^+ cofactor binding and the second one providing the groups for substrate fixation and reaction [28]. Cross-linking enzymes after their immobilisation in/on a support [41, 42] or their aggregation [43] is a classical strategy used for their stabilisation. Cross-linking with GA mainly proceeds via lysine residues of the enzyme. A lysine-rich inert protein, typically BSA, may be added to limit intramolecular reactions [44]. Coating with ionic polymers may also be used to protect enzymes against denaturation. Polyethyleneimine, a polycationic polymer bearing ionised amino groups, is able to interact with anionic groups located at the protein surface in two or more subunits, resulting in enzyme stabilisation [45]. Very similarly, Nafion is a cation exchange polymer composed of three different areas: hydrophilic cluster of sulfonic groups with diameters from 30 to 50 nm, hydrophobic semi-crystalline region which is a Teflon-like backbone and a zone of intermediate hydrophobicity. The high density of sulfonates enables interactions of the polymer with any region of the protein surface bearing cationic groups. It was shown that higher activity could be achieved for urease immobilised in Nafion than for the same enzyme cross-

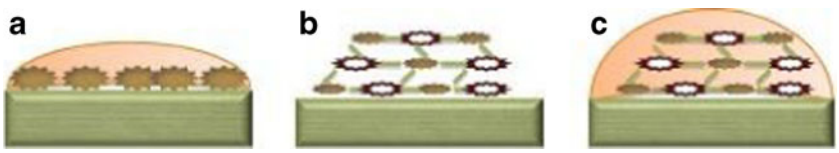

Fig. 1 Schematic representation of the three modes of enzyme immobilisation tested. **a** Biomembrane coated with Nafion, **b** cross-linking with GA, **c** combined method

linked with GA [30]. Another advantage of Nafion polymer used for biosensor fabrication is that it acts as a selective barrier towards anionic interfering substances and as a protective layer against electrode fouling. However, active lifetimes of dehydrogenases in acidic media generated within Nafion membranes may be limited [45]. Different strategies, including Nafion modification [46] or rigidification of enzyme structure, e.g. via cross-linking before Nafion coating [32, 33], have been reported to solve this problem.

In this study, three methods were compared for PFDH immobilisation. In each case, PFDH solution was dropped onto the transducer and allowed to dry. Then, (1) the biomembrane was coated with Nafion, or (2) PFDH enzyme was cross-linked for 30 min using GA vapours, or (3) PFDH was cross-linked with GA and covered with a Nafion membrane (Fig. 1). Each protocol was repeated twice, and the six biosensors were tested for 10 mM FA. No variation of conductance was observed following FA addition when PFDH was directly coated with Nafion (data not presented), confirming adverse effects of the polyanionic polymer on PFDH activity. In contrast, FA injection into the measurement cell caused a significant increase of conductance when the two other modes of immobilisation were used (Fig. 2), network formation through PFDH cross-linking playing, as expected, a positive role against enzyme denaturation and desorption from the surface.

As depicted in Fig. 2, the steady-state response was achieved within 25 min in absence of Nafion and about twice more slowly when Nafion was added over the cross-linked PFDH biofilm. This difference may be attributed to the creation of an additional diffusion barrier hindering substrate access to the enzyme and slowing down the equilibration process between ions generated in the biomembrane and electrolyte ions. The signal, but also the background noise, was doubled with Nafion. It may be assumed that H^+ ions generated through the reaction are retained within the membrane, causing a local increase of conductance, and then an increase of biosensor signal and noise. Similar results had been already observed by Soldatkin et al. using the same transducer but with glucose oxidase instead of PFDH [47].

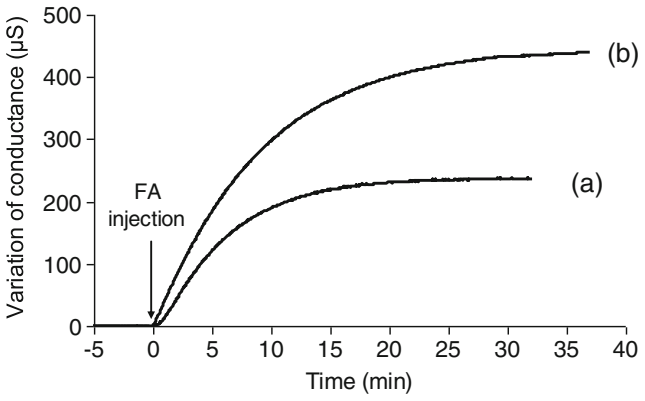

Fig. 2 Influence of immobilisation mode on biosensor response. Cross-linking for 30 min without (**a**) and with (**b**) Nafion coating. The 0.045 mg PFDH and 13 µg NAD^+ co-immobilised in the biomembrane, [FA]=10 mM. Measurement in phosphate buffer pH 7 at T=23 °C±2 °C

The third mode of immobilisation combining cross-linking and coating with Nafion was finally selected to achieve the best sensitivity.

Optimisation of enzyme and co-enzyme parameters

To maximise biosensor performances, we next optimised different parameters affecting enzymatic reaction.

Enzyme loading

The behaviour of immobilised enzymes may be, in many aspects, different from that of free enzymes in solution. In particular, it has been shown that loading enzymes on a support can increase or decrease the retained activity, depending on the nature and properties of the protein. In some cases, enzyme overcrowding reduces activity as a consequence of spatial restrictions, decrease of active site accessibility or protein denaturation. The reverse behaviour has been also observed for some flexible proteins [48]. In this work, the influence of PFDH amount in the biomembrane was investigated in the 0.015–0.045 mg range. For that, a 150 g L^{-1} PFDH biomembrane solution containing 50 mg mL^{-1} BSA, 100 mg-mL^{-1} glycerol and 6.5 % NAD^+ was prepared and different volumes of this solution (0.1, 0.2 or 0.3 µL) were pipetted on the sensors. Two sensors were fabricated at each enzyme amount level. PFDH concentration was limited to 150 g L^{-1} to avoid enzyme precipitation. Likewise, the maximal volume deposited on the electrodes was 0.3 µL owing to the low dimensions of the sensitive parts of the transducers. As depicted in Fig. 3, the initial reaction rate as well as the steady-state variation of conductance obtained for 10 mM FA increased almost linearly with PFDH amount. The highest values were obtained for 0.045 mg enzyme. No crowding effect seemed to occur at this value, and it was therefore decided to select this mass of protein for subsequent experiments.

Cofactor parameters

All previous experiments were performed by co-immobilising a large amount of NAD^+ with PFDH, as proposed in references [12] [19]. The cofactor was not regenerated and not added in solution. However, a rapid decrease of biosensor signal was observed in those conditions (Fig. 4). Fifty percent of the initial signal was lost after the second measurement, 80 % after the third. This result was attributed to NAD leakage from the biomembrane. In contrast, feeding the cofactor in solution at each FA measurement generated reproducible signals over a 12-day period (Fig. 4). Increasing NAD^+ concentration from 20

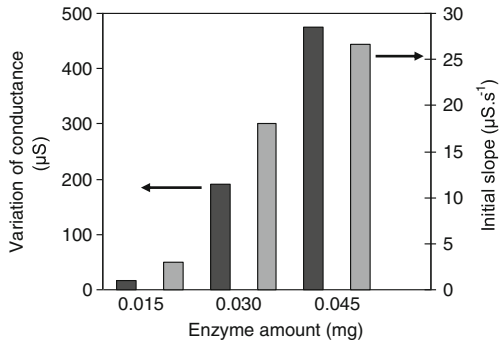

Fig. 3 Influence of PFDH amount on biosensor signal (*pale grey*) and initial slope of biosensor response (*dark grey*). The 13 µg NAD^+ co-immobilised with PFDH in the biomembrane cross-linking with GA for 30 min and coating with Nafion membrane. Measurement conditions were identical to that of Fig. 2

to 80 µM did not enhance biosensor signal, neither at 5 nor at 110 mM FA (data not shown). A 20 µM NAD^+ concentration was therefore added in solution thereafter.

Optimization of membrane parameters

Cross-linking step

Different parameters, such as temperature, GA concentration and exposure time, may affect cross-linking reaction and, therefore, the stability and permeability of the resulting biomembrane [44]. Considering literature data and for practical reasons, reaction was performed at room temperature using GA vapours generated from a commercial 25 % aqueous solution [38, 44, 49]. Only reaction time varied. Three biosensors were prepared, setting the exposure times at 10, 15 and 30 min, respectively. Biosensor response was recorded for 10 mM FA over a 24-day period. As seen in Fig. 5a, the lowest signal was obtained at the shortest time of reaction (10 min), which may be attributed to biomaterial leakage due to insufficient cross-linking. The 15-min exposure biosensor exhibited higher initial response but also higher instability (Fig. 5b). In this case, it may be hypothesised that the structure of the polymeric network created via cross-linking process is tighter, limiting PFDH release. However, enzymes are likely to remain relatively flexible within the immobilisation matrix. Irreversible conformational changes may therefore occur in course of the successive measurements, causing a progressive inactivation, and thus a decrease of the biosensor signal with time. Improvement of signal stability by increasing exposure time to 30 min was consistent with the decreased flexibility of FDH induced by higher cross-linking degree. Therefore, a 30-min exposure time was selected for further experiments. An optimal reaction time of 5 min had been reported for acethylcholinesterase cross-linking at higher temperature (37 °C) by Li et al. [49].

Nafion solution volume

In the following series of experiments, four biosensors were prepared by dropping enzyme solution onto the electrodes as described in the "Experimental" section and depositing variable volumes of Nafion solution (0.1, 0.2, 0.3 or 0.4 µL) after cross-linking. All steps of electrode fabrication were characterised by impedance spectroscopy and the biosensor responses to 2 mM FA were measured by conductometry. As depicted in Electronic Supplementary Material Fig. S1, an increase of interfacial admittance was observed after each step of sensor construction. This could be assigned to the fact that charged species are

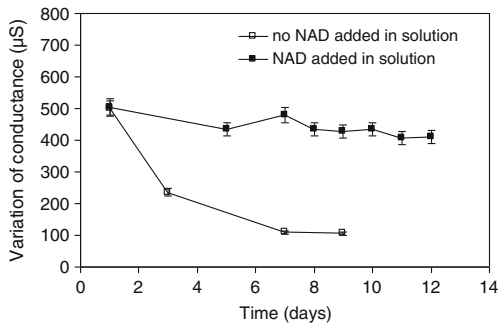

Fig. 4 Effect of 20 µM NAD^+ supplementation in measurement solution on biosensor signal stability. The 0.045 mg PFDH and 13 µg NAD^+ co-immobilised in the biomembrane. Other conditions were identical to that of Fig. 3

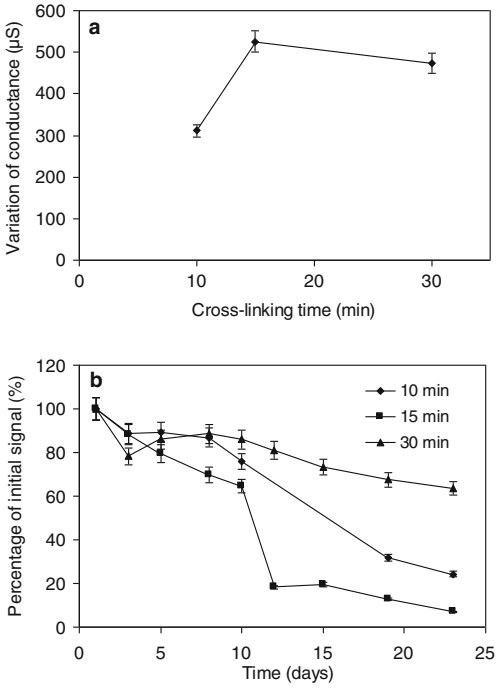

Fig. 5 Influence of cross-linking time on biosensor signal (**a**) and stability (**b**). The 0.045 mg PFDH and 13 µg NAD^+ co-immobilised in the biomembrane. The 20 µM NAD^+ was added into solution. Other conditions were identical to that of Fig. 3

added, BSA and PFDH (first layer) as well as Nafion polymer (second layer) being negatively charged at neutral pH. The surface modification may also induce electrode (at least partial) passivation. Variations of the real part of admittance at 40 kHz also increased with Nafion volume up to 0.3 μL and decreased beyond this value (Fig. 6a), indicating that conductive properties of the membrane layer were maximal for a 0.3 μL deposit.

In a similar way, biosensor signal recorded for 2 mM FA increased with Nafion volume up to 0.3 μL (Fig. 6b), which could be attributed to enhanced protons accumulation within the membrane [47]. On the contrary, diffusion through the membrane and, therefore, response time was not strongly affected by varying its thickness in the range investigated (Fig. 6b). Therefore, 0.3 μL Nafion was chosen for further experiments.

Optimisation of measurement parameters

Ionic strength and buffering capacity

Ionic strength and buffering capacity are important parameters that may affect the conductometric biosensor response by modifying electrical properties of the electrode/electrolyte interface, local ion concentrations and ion mobilities. In this study, ionic strength was modified by adding 5 to 120 mM NaCl to the measurement medium composed of 5 mM phosphate buffer, or by increasing phosphate buffer concentration from 2 to 100 mM, which increased buffering capacity at the same time. A sharp decrease of the biosensor response was observed by increasing phosphate buffer (Fig. 7a) or NaCl (Fig. 7b) concentration. However, the conductance drop was slightly less pronounced in presence than in absence of Nafion (Fig. 7b).

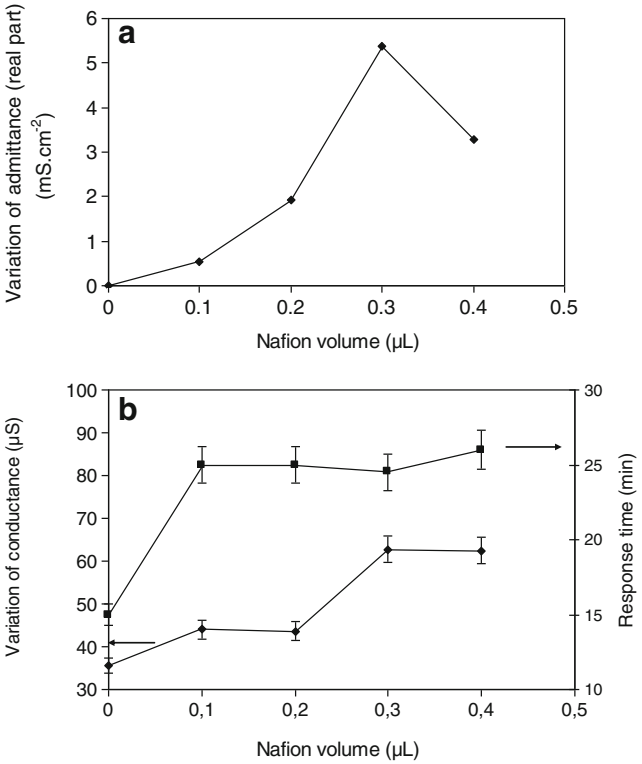

Fig. 6 Influence of Nafion solution volume on the real part of membrane admittance at 40 kHz (**a**), and on biosensor signal (*closed diamond*) and response time (*closed square*) (**b**). Applied potential for impedance measurements, 10 mV. Conditions for biosensor response measurements, [FA]=2 mM. 20 μM NAD^+ added in solution. Other conditions were identical to that of Fig. 3

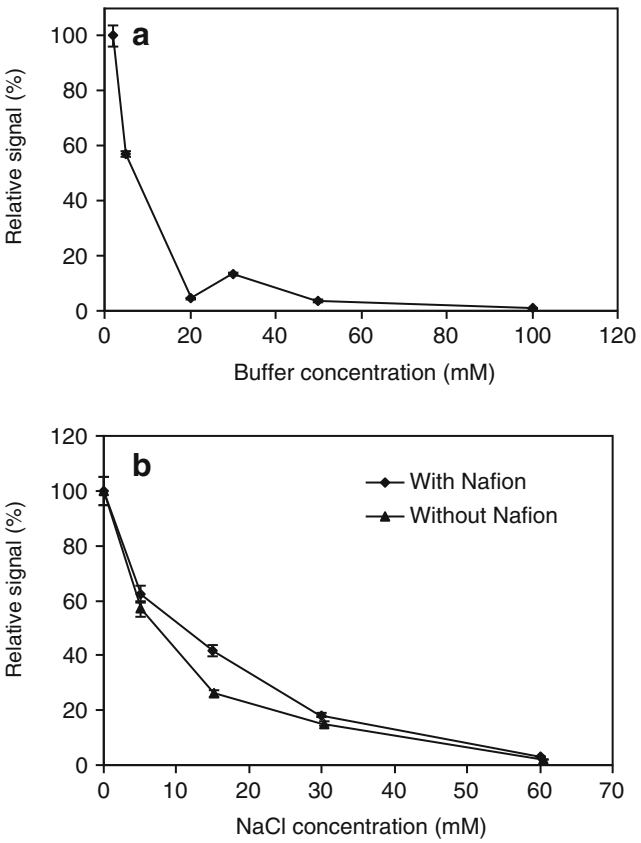

Fig. 7 Influence of buffer (**a**) and NaCl (**b**) concentration on biosensor response. Measurements presented in Fig.7a are performed in presence of Nafion. [FA]=5 mM. The 20 μM NAD^+ was added into solution. Other measurement conditions were identical to that of Fig. 2

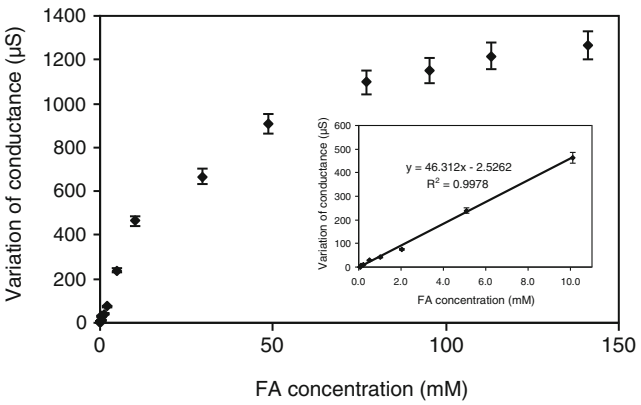

Fig. 8 Influence of FA concentration on the conductometric biosensor signal. The linear part of the *curve* is presented in the *insert*

Table 1 Analytical characteristics of some FDH- and AOX-based FA biosensors

Transduction	Enzyme	Electrode	Immobilisation	Linear range (mM)	LOD (μM)	Samples	Ref
Conductometry	PFDH	Au IDE	GA cross-linking+Nafion	0.05–10	18	Tap water, river water	This work
	PFDH	Au IDE	GA cross-linking	10–200	–	Formidron, Descoton forte, formalin	[12]
	HansFDH			25–200			
	AOX	Au IDE	GA cross-linking	0.05–500	50	Formidron, Descoton forte, formalin	[23]
Amperometry	HansFDH	Platinized graphite	Entrapment in Os-containing redox polymers+Nafion	0.027–20	3	Formidron, Descoton forte, formalin	[11]
	PFDH	Glassy carbon	Entrapment into mesoporous silica	0.004–1	1.2	–	[15]
	PFDH	Screen-printed carbon modified with MWCNTs	–	0.0001–1.5	0.1	Release medium of human cells treated with anti-cancer prodrugs	[16]
	PFDH	Au	Enzyme mixed with Nafion	0.03–0.3	0.5	Mackerel fish	[21]
	AOX	Platinized screen-printed carbon	GA cross-linking	0.06–0.46	60	Commercial samples for histology	[24]
Potentiometry	AOX	H^+-sensitive screen-printed Ag/AgCl	Covalent binding to acrylic microspheres	0.3–316	300	Shrimp	[22]

HansFDH FDH from *H. polymorpha*

Similar results had been already reported for other enzyme conductometric biosensors [47, 50]. Results observed in Fig. 8a and b might be partly attributed to a decrease in conductometric transduction efficiency with increased background conductivity (polarisation effect). In addition, buffer phosphate ions, by associating with protons generated at the electrode surface, act as a carrier and facilitate their transport from the enzyme biomembrane to the solution [44, 45]. An increase of buffer concentration is therefore expected to decrease the free H^+ local concentration at the electrode and, therefore, the biosensor steady-state response. Adding the negatively charged groups of Nafion creates a barrier to the diffusion of chloride or phosphate anions, reducing the contribution of the "carrier-mediated" mechanism and favouring protons diffusion out of the membrane in exchange with Na^+ buffer counter-ions. Increasing NaCl concentration in presence of the sulfonated polymer results in higher proton flux and then in a decrease of biosensor signal, but in a lesser extent than in absence of Nafion.

The highest signal was therefore obtained in presence of Nafion and at the lowest phosphate concentration tested (2 mM). However, the sensitivity to small changes in conductivity was higher at this concentration, decreasing repeatability. A 5 mM phosphate buffer was then considered as optimal to achieve the best compromise between intensity and reproducibility.

pH

To determine optimal pH to be used for measurement, three biosensors were fabricated, and the evolution of the response towards a 5 mM FA solution was studied by varying buffer pH in the 5–9 range. As seen in Fig. S2 (Electronic Supplementary Material), the highest signal was achieved at pH 7.1, relatively close to the optimal working pH of 7.8 or 8.9 reported for free PDFH [51, 52]. The difference may be attributed to a change in enzyme conformation in course of immobilisation process and/or to the modification of ionic strength accompanying pH variations. These hypotheses are of course to be confirmed.

Table 2 FA recovery from spiked water samples (n=3)

Sample	Added (mM)	Found (mM)	Recovery (%)
Tap water	0.20	0.21	105
	0.42	0.40	95
	0.83	0.87	105
Rhone river water	0.19	0.21	110
	0.32	0.32	100
	0.42	0.43	102
	0.84	0.80	95

Analytical characteristics of PFDH-based conductometric biosensor

Analytical characteristics of PFDH-based conductometric biosensor were evaluated in the optimal conditions previously defined using 14 standard solutions in the 0.06 to 140 mM range. Three replicates were performed at each concentration level. As seen in Fig. 8, biosensor response was linear up to 10 mM FA and increased progressively to achieve a plateau at about 150 mM FA. Correlation coefficient and sensitivity were 0.998 and 46 μS mM^{-1}, respectively. The detection limit, calculated as described in the "Experimental" section, was 18 μM corresponding to 3 ppb atmospheric concentration. This value is far below the human olfactory perception limit (0.5 to 0.83 ppm) as well as the short-term (30-min) guideline of 100 ppb recommended for indoor air quality by the World Health Organization. This is also of the same order as the values already reported for conductometric or potentiometric biosensors (Table 1).

The short-term reproducibility of the biosensor response was tested at three concentration levels in the 1–10 mM range. The variation coefficient obtained from five measurements performed within 1 day with the same sensor was very good as it was lower than 5 % in the concentration range studied.

To test the biosensor storage stability, one sensor was measured two to three times a week over a 24-day period. Meanwhile, the biosensor was stored at +4 °C in 20 mM phosphate buffer pH 7. As shown previously (Figs. 4 and 5b), the biosensor was stable for 12 days and decreased thereafter, about 65 % of the initial signal being recovered after 24 days.

Recovery experiments were further performed in one tap water sample and one water sample from the Rhone River (France). Samples were spiked with different concentrations of FA and passed through Chromabond ion exchange cartridges. Then, 20 μL of the spiked samples were injected in the measurement cell and analysed for their FA content using the proposed biosensor. Three replicate measurements were performed for each sample. No FA could be detected in the samples before spiking, while excellent recoveries (95–110 %) were obtained for spiked samples (Table 2).

Conclusion

In this work, a novel conductometric biosensor based on formaldehyde dehydrogenase from *P. putida* was proposed for formaldehyde determination in water samples. Formaldehyde oxidation catalysed by PFDH produces formate ions and protons detectable by conductometry. Three modes of immobilisation were evaluated, including cross-linking of the enzyme, coating with Nafion sulfonated fluoro-copolymer and a combination of both methods. The latter protocol was the most efficient. The Nafion membrane was able to concentrate NAD^+ cofactor in the vicinity of PFDH enzyme and to induce a local H^+ concentration effect, allowing significant signal enhancement. In the optimal measurement conditions (phosphate buffer 5 mM, pH 7), the calculated limit of detection was 18 μM of FA, corresponding to 3 ppb atmospheric concentration. This value is far below the short-term (30-min) guideline of 100 ppb recommended for indoor air quality by the World Health Organization. The signal was linear up to 10 mM and stable over a 12-day period when the biosensor was stored at 4 °C in phosphate buffer 20 mM pH 7 between two measurements. Very good recoveries (95–110 %) were obtained for tap water and river water samples spiked with FA, demonstrating the applicability of the biosensor for the analysis of real samples.

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